Copper Metabolism in Mottled Mouse Mutants

COPPER THERAPY OF BRINDLED (Mo^{br}) MICE

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Copper therapy was applied to brindled mouse mutants, which suffer from lethal hypo-cupraemia, by using cuprous and cupric solutions. The method of treatment was a single subcutaneous injection of 50 µg of copper at 7 days of age. Early effects of the dose were: prevention of the tremors and spasms seen in untreated mutants, raising to normal and near-normal of caeruloplasmin oxidase and lysyl oxidase activities and pigmentation of skin and fur. Growth of mutants was retarded up to 23 days of age, but thereafter they rapidly gained weight to be nearly normal by 60 days of age. At 3 days after injection, copper concentrations in previously deficient mutant organs apart from liver were at least as much as those of treated normals, which had remained unchanged. Copper in mutant livers had increased only slightly in comparison with the normal control. A state of copper deficiency recurred in mutant tissues by 25 days after injection. A solution of Cu$^+$, retained as such by an alkyl polyether, and sebacic acid resulted in greater growth rates after 23 days than did three other copper treatments. Cu$^+$ may have resulted in an improved growth response owing to it being more readily metabolized than Cu^{2+}. Delayed release of copper from the site of injection may have played an important role.

Mottled mice provide an animal model of the congenital copper-deficiency disease, Menkes' syndrome, and are excellent for studies of copper metabolism (Hunt, 1974, 1976; Camakaris *et al.*, 1979).

Previous reports of copper therapy in brindled males (Mo$^{br/y}$) have demonstrated increased survival up to 28 days of age, but only moderate improvement in weight gain (Hunt, 1976). Up to now, copper therapy of infants with Menkes' syndrome has proved of little benefit, if Cu–histidine, Cu–EDTA or Cu–albumin complexes were used. However, treatment in these cases was not usually commenced until at least 2 months after birth, and some irreparable damage may have occurred (Danks, 1977). Henkin (1978) has reported patients treated from the first days or weeks of life with oral Cu–nitriloacetate with very variable clinical results; none of the patients were normal; penetration of copper to the brain was particularly poor.

The present paper describes results of various forms of copper therapy of brindled (Mo$^{br/y}$) mice and reports survival to 10 months with normal growth and fertility in treated males.

Experimental

The mouse strain used possessed the lethal mutant gene brindled (br), an allele at the mottled locus (Fraser & Sobey, 1953). The affected male mice (Mo$^{br/y}$) die at around 14 days *post partum*. Adult mice were fed on Mecon rat and mouse cubes *ad libitum* containing 16.7 µg of copper/g dry wt., and acidified water containing 2.5 µg of copper/ml and were housed in plastic boxes with steel tops.

Mice were treated with one of eight copper solutions (Table 1). Alkyl polyether is a compound capable of retaining copper in its reduced state (Cu$^+$), and was kindly supplied by I.C.I. (Australia) Ltd. Research Department, Newson Street, Ascot Vale, Vic., Australia. This substance is toxic in large doses, so was diluted to 10% (v/v), at which it is still capable of retaining Cu$^+$. The Cu$^+$ in this solution precipitates if exposed to water, so the diluent chosen was propane-1,2-diol, which is non-toxic and sufficiently non-viscous for injection. Alkyl polyether was shown to retain at least 99% Cu$^+$ by polarographic, n.m.r. and iodometric techniques (E. G. Walliczek, unpublished work). Sebacic acid was added to some of

Table 1. *Copper solutions used in therapy*

Form of copper salt in solution	Complexing or chelating species (equimolar to copper)	Amount of alkyl polyether (v/v)	Solvent	Abbreviation
Cu^+ chloride	Sebacic acid	10 %	Propane-1,2-diol	$Cu^+Seb(p)$
Cu^{2+} chloride	Sebacic acid	10%	Propane-1,2-diol	$Cu^{2+}Seb(p)$
Cu^+ chloride	—	10%	Propane-1,2-diol	$Cu^+(p)$
Cu^{2+} chloride	—	—	Propane-1,2-diol	$Cu^{2+}(p)$
Cu^{2+} chloride	EDTA	—	Water	Cu–EDTA (aq.)
Cu^{2+} chloride	Histidine	—	Water	Cu–histidine (aq.)
Cu^{2+} chloride	—	—	Water	$CuCl_2$(aq.)
Cu^{2+} sulphate	—	—	Water	$CuSO_4$ (aq.)

Table 2. *Survival after different treatments of mutants at 7 days*
The number of mice injected is followed by the number of mice that survived the injection to reach 60 days of age.

Copper solution	Dosage of copper (μg) ...	50		25		12.5		6.25	
		Injected	Survived	Injected	Survived	Injected	Survived	Injected	Survived
$Cu^+Seb(p)$		9	8	6	3	4	1	3	0
$Cu^{2+}Seb(p)$		7	6	1	0	4	0	2	0
$Cu^+(p)$		9	6	—	—	—	—	—	—
$Cu^{2+}(p)$		13	11	6	2	4	0	3	0
Total		38	31	13	5	12	1	8	0
Survival (%)		82%		38%		8%		0%	

the solutions, as it can complex copper (E. G. Walliczek, unpublished work) and it has properties that would facilitate passage through cell membranes. As it can be absorbed percutaneously, other work is required on this mode of copper administration.

Copper was administered by a single injection of 10μl of solution subcutaneously in the midline dorsally to mice aged between 7 and 12 days, the amount of copper in the solution being adjusted to provide the desired dosage. Injections were made with a 10μl SGE syringe. Various doses of copper were given initially in order to establish the amount needed to give maximum success in treatment. Dosages used were 200, 100, 50, 12.5 and 6.25μg.

In determining growth curves, mice were weighed every 3 days after injection up to 60 days of age. Only males were treated and weighed. Litter size was kept at four mice to eliminate growth differences owing to differing litter size, and to allow treated mutants easy access to maternal milk to aid their growth. Each litter contained two or three mutant males and one treated or untreated normal mouse to allow within-litter comparisons of different treatments.

Mice were killed at the ages indicated and copper concentrations of various organs were measured by the method of Stevens (1972). Copper was assayed by using the Varian-Techtron CRA-90 atomic absorption spectrophotometer, containing the BC-6 background-subtract module. Caeruloplasmin was assayed by the method described in the preceding paper (Camakaris *et al.* 1979).

Results

Influence of different copper solutions used

These are listed in Table 1. Administration of 50μg of copper as Cu–EDTA or Cu–histidine proved very toxic to the young mice, resulting in death of both normals and mutants in less than 24h after injection. In the form of $CuSO_4$ (aq.), 50μg of copper was also toxic to mutants, resulting in nervous-system damage evident by gross ataxia, whereas an equivalent dose of $CuCl_2$ (aq.) or of the other four remaining copper solutions caused no such symptoms. Subsequent therapy was therefore restricted to $Cu^+Seb(p)$, $Cu^{2+}Seb(p)$, $Cu^+(p)$ and $Cu^{2+}(p)$ solutions (Table 1).

Choice of dose and age of administration

Table 2 shows that a 50μg injection of copper was more effective than the smaller doses, resulting in the most frequent survival of mutants up to 60 days of age. With doses of 200μg and 100μg, growth stunting and nervous-system damage were frequent in mutants. Administration of 50μg of copper at 9 days or later resulted in a decreased survival of mutants; the later the injection, the less successful the treat-

Table 3. *Copper contents of tissues 3 days after injection*

Data for untreated mice are taken from Camakaris *et al.* (1979). Results of all copper-treated normal mice were combined, since the form of copper used did not alter the results significantly. Tissue copper concentration is expressed as μg/g dry wt. Serum copper (μg/100 ml) and caeruloplasmin (A_{250} units) measurements were made on pooled samples from three mice. Urine copper is μg/mmol of creatinine. Results are means ± s.d. with the numbers of observations in parentheses. The total copper (μg) in liver, kidneys and skin for these observations are given in the lower segment of the Table.

| | Treatment | | | | | |
| | Mo$^{br/y}$ | | | | Mo$^{+/y}$ | |
Mouse ...	Cu+Seb(p)	Cu$^+$(p)	Cu^{2+}(p)	Untreated	Treated	Untreated
Tissue						
Brain	4.3 ± 0.47 (8)	3.6 ± 0.37 (3)	3.8 ± 0.61 (8)	3.2 ± 0.38 (6)	9.5 ± 1.45 (9)	12.0 ± 2.50 (6)
Liver	85.3 ± 23.70 (8)	85.1 ± 8.40 (3)	31.8 ± 13.50 (7)	13.3 ± 1.12 (5)	590.4 ± 166.20 (9)	169.2 ± 92.50 (6)
Kidney	166.3 ± 69.70 (4)	170.8 ± 13.90 (3)	182.7 ± 38.00 (4)	49.4 ± 9.60 (6)	13.6 ± 1.65 (7)	15.6 ± 1.26 (6)
Lung	21.4 ± 5.90 (4)	22.8 ± 6.01 (2)	16.9 ± 6.20 (4)	8.8 ± 2.40 (3)	9.2 ± 1.58 (5)	7.3 ± 0.49 (3)
Spleen	13.6 ± 3.80 (6)	16.2 ± 3.50 (3)	11.2 ± 3.60 (6)	4.1 ± 0.71 (5)	17.6 ± 5.06 (9)	16.5 ± 4.04 (5)
Muscle	12.9 ± 6.80 (6)	10.5 ± 0.56 (3)	6.9 ± 0.62 (6)	4.3 ± 1.05 (6)	8.2 ± 1.71 (9)	8.7 ± 1.20 (6)
Skin	30.5 ± 6.40 (4)	—	20.8 ± 8.50 (3)	2.6 ± 1.05 (4)	13.3 ± 4.70 (3)	4.1 ± 0.43 (4)
Serum	58.0	—	62.5	24.0	55.5	76.0
Caeruloplasmin	0.271	—	0.321	0.072	0.252	0.361
Urine	146.4 (2)	—	—	59.5	96.2 (2)	66.4
Total copper (μg)						
Liver	3.67	4.81	1.49	0.66	26.6	10.40
Skin	10.00	—	8.50	1.08	7.40	1.83
Kidneys	2.70	3.30	3.30	0.92	0.23	0.30

Table 4. *Copper contents of tissues 11, 25 and 53 days after treatment*

All mice were treated with $Cu^+Seb(p)$. Serum copper ($\mu g/100ml$) and caeruloplasmin (A_{250} units) measurements of mice 11 days after treatment were made on pooled samples from two mice. The total copper in liver, kidneys and skin for these observations are given below. Tissue copper concentrations are expressed as means ($\mu g/g$ dry wt.) followed by the number of observations in parentheses.

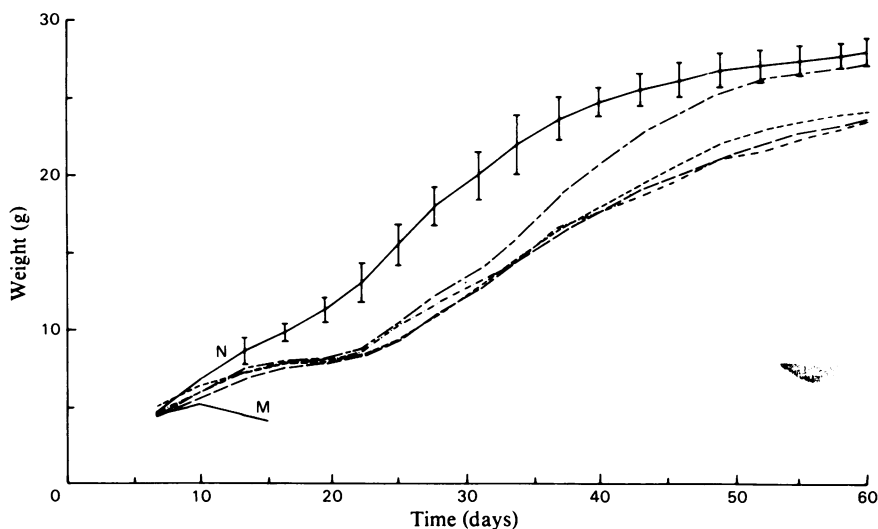| Time after injection (days) ...	11			25			53	
Tissue	Treated $Mo^{+/y}$	Untreated $Mo^{+/y}$	Treated $Mo^{br/y}$	Treated $Mo^{+/y}$	Untreated $Mo^{+/y}$	Treated $Mo^{br/y}$	Untreated $Mo^{+/y}$	Treated $Mo^{br/y}$
Brain	13.3 (2)	8.8 (2)	3.9 (2)	15.1 (2)	17.5 (3)	6.0 (4)	21.3 (2)	9.2 (2)
Liver	417.5 (2)	61.2 (2)	13.0 (2)	11.0 (2)	18.9 (3)	11.1 (4)	16.6 (2)	17.8 (2)
Kidney	14.6 (2)	11.9 (2)	168.8 (2)	17.9 (2)	20.0 (3)	149.0 (4)	19.8 (2)	76.5 (2)
Lung	11.5 (2)	12.3 (2)	16.4 (2)	—	13.5 (3)	15.2 (2)	18.3 (2)	15.6 (2)
Spleen	11.1 (2)	11.1 (2)	8.5 (2)	10.4 (2)	14.5 (3)	3.8 (2)	7.6 (2)	5.3 (2)
Muscle	6.6 (2)	7.0 (2)	7.7 (2)	6.0 (2)	6.0 (3)	3.4 (2)	7.4 (2)	6.7 (2)
Skin	4.7 (2)	4.0 (2)	1.9 (2)	5.3 (2)	7.0 (3)	5.3 (2)	3.8 (2)	3.5 (2)
Serum	126.0	90.0	70.5	67.0 (2)	86.3 (2)	33.0 (2)	78.0 (2)	47.5 (2)
Caeruloplasmin	0.344	0.387	0.195	0.288 (2)	0.410 (2)	0.118 (2)	0.336 (2)	0.066 (2)
Total copper (μg)								
Liver	29.50	4.22	0.74	5.10	5.10	2.10	1.80	1.70
Skin	4.20	5.30	2.10	6.80	—	2.60	—	—
Kidneys	0.40	0.31	3.80	2.20	1.60	8.30	3.60	10.40

Fig. 1. *Weight gain of mice after subcutaneous injection of 50 µg of copper at 7 days*
The curve labelled 'M' is the mean growth curve for untreated mutant mice (observations on three mice). The curve labelled 'N' is the mean growth curve for untreated and treated normal mice (observations on nine mice): S.D. values are indicated by the vertical bars. Designations for growth curves of treated mutant mice are as follows (with numbers of mice in the group in parentheses): —————, $Cu^+Seb(p)$ (8); —·—·—·—, $Cu^+(p)$ (6); —————, $Cu^{2+}Seb(p)$ (6); ————, $Cu^{2+}(p)$ (11); Student's t test was used to assess significance. The P values were as follows: normals versus $Cu^+Seb(p)$-treated mutants, $P < 0.05$ day 7 to day 49, $P > 0.05$ day 49 to day 60; $Cu^+Seb(p)$-treated mutants versus $Cu^{2+}(p)$-treated mutants, $P > 0.05$ day 7 to day 37, $P < 0.05$ day 37 to day 60; normals versus $Cu^+(p)$- or $Cu^{2+}Seb(p)$- or $Cu^{2+}(p)$-treated mutants, $P < 0.05$ day 13 to day 60.

ment. Therefore, subsequent treatment was restricted to 50 µg of copper administered at 7 days of age.

Early effects

Local irritation appeared around the site of injection in both normals and mutants by 24 h. This formed a sore which regressed to form a patch of scar tissue by adult age.

All four solutions prevented the tremors and spasms seen in untreated mutants. Strong pigmentation appeared over most parts of the skin by 24 h after treatment, and subsequent hair growth was dark grey. New hair growing approx. 7 days after injection was pale, and, by 30 days of age the treated mutants were pale grey, and remained so throughout life. The kinky whiskers gradually straightened until approx. 25 days after injection, but then regressed to their former state by 30–40 days of age. At 3 days after injection, caeruloplasmin oxidase activity was normal (Table 3) and lysyl oxidase activity in urea extracts of skin (Rowe *et al.*, 1977) was near normal (Royce *et al.*, 1978), these enzymes being severely deficient in untreated mutants. However, caeruloplasmin oxidase activity had fallen by 11 days after injection and was very low by 32 days (Table 4). Urinary excretion of copper was high in mutants 3 days after injection (Table 3).

Effect on growth and survival

Fig. 1 shows that the weight gain in mutants was very slow for as long as 23 days after treatment with any of the four solutions, but then mutants put on weight at a faster rate than normals (Fig. 2). Mutants treated with $Cu^+Seb(p)$ showed significantly better catch-up growth than those in the other three treatment groups, reaching near-normal weight by 60 days (Fig. 1). Their rapid growth phase began earlier than that of the other treatment groups, except those with $Cu^+(p)$, and there was an extra growth spurt at 34–47 days (Fig. 2). Growth of normal mice was unaffected by treatment, and these have been grouped together in the graphs.

This weight gain was a true catch-up growth phenomenon involving all parts of the body (Table 5). The mutant adult males are healthy and vigorous (more so than viable blotchy mutants), and the first mice treated in our laboratory have now survived to 10 months of age. Apart from having pale-grey fur and kinky whiskers, they appear and behave normally, and are also fertile. Mutant females ($Mo^{br/br}$) produced from matings between treated mutant males ($Mo^{br/y}$) and heterozygous females ($Mo^{br/+}$) have also been treated, with similar response to treated males, and have also proved fertile, producing live offspring.
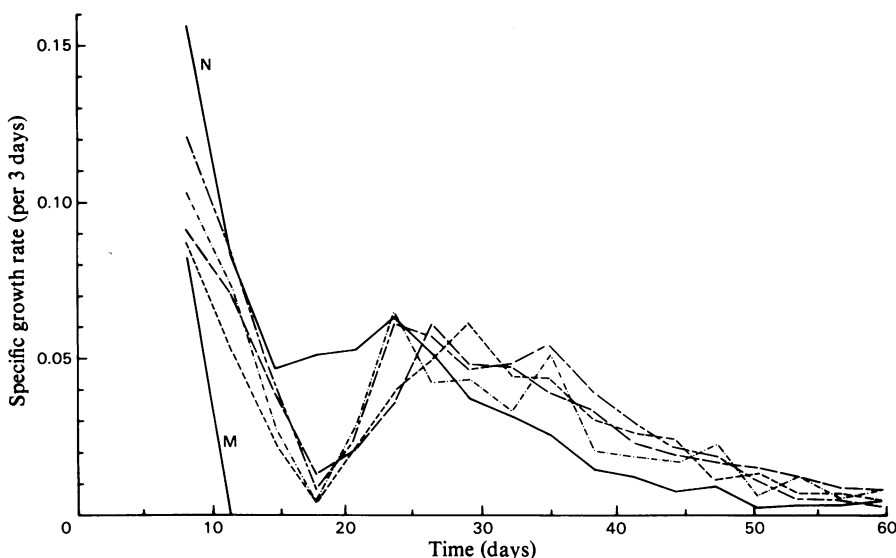
Fig. 2. *Specific growth rates of mice after subcutaneous injection of 50 μg of copper at 7 days*
The curve labelled 'M' is for untreated mutant mice (observations on three mice). The curve labelled 'N' is for untreated and treated normal mice (observations on nine mice). Designations for curves for treated mutant mice are as follows (with numbers of mice in the group in parentheses): —·—·—, $Cu^+Seb(p)$ (8); —·—·—, $Cu^+(p)$ (6); ————, Cu^{2+}-$Seb(p)$ (6); ----, $Cu^{2+}(p)$ (11).

Table 5. *Body-weight analysis of normal and mutant adults*
Measurements were made on one 60-day-old normal and one 60-day-old mutant mouse that were litter-matched. Total weights: normal, 25.91 g; mutant, 24.11 g.

| Body part | Per cent of total body wt. | | Body part | Length (mm) | |
	$Mo^{+/y}$	$Mo^{br/y}$		$Mo^{+/y}$	$Mo^{br/y}$
Viscera	31.22	29.29	Tail	78	87
Skin	15.61	13.76	Rump to neck	60	64
Lean carcass	53.17	56.95			

Organ distribution of copper

In normal mice the liver copper concentration was greatly elevated 3 days after injection and remained thus 11 days after injection (Tables 3 and 4). Copper concentrations in other tissues did not change. In the mutants, tissues other than liver changed from an originally copper-depleted state to build up concentrations of copper similar to or greater than normal, whereas the liver content reached only half that found in untreated normal mice, and was much lower than in the treated normal mice. Increase in liver copper content in mutants was greater with Cu^+ than with Cu^{2+} (Table 3). Three heterozygote mice were treated in an equivalent way with $Cu^{2+}(p)$, and the liver copper concentrations 3 days after injection were 234.9, 120.3 and 286.2 μg/g dry wt. (normal range for heterozygotes of 11 days of age, 24.62 ±

2.88). These values were intermediate between those attained by normals and mutants. The percentages of the administered dose found in the livers of normal, mutant and heterozygote mice 3 days after injection were 31.7%, 4.7% and 22.6%. Brain concentrations in the mutants remained very low at all stages after treatment.

Copper concentrations in most tissues fell gradually as time passed (Table 4). These values must be interpreted against the background of the age-related changes in normal mice, which were especially marked in the liver. By 25 days after injection, concentrations in brain, spleen, muscle, plasma and caeruloplasmin were lower in mutants, whereas the values in kidney were still increased and those in liver were only a little lower than normal. By 53 days after injection only brain, plasma and caeruloplasmin

values were low, values in liver and most other tissues were close to normal, and kidney values remained very high. Also at this stage, cells from the duodenum, jejunum and ileum of treated mutants contained normal amounts of copper, in contrast with 11-day-old untreated mutants, which had very high concentrations in the small intestine (Camakaris et al., 1979).

Discussion

These experiments show that it is possible to achieve long-term survival, near-normal growth and fertility in both affected males and homozygous affected females for the brindled mutation with a single dose of subcutaneous copper administered at day 7. This will allow considerably more investigations of the effects of this mutation to be carried out. It is indeed surprising that a single dose has produced such a long-term benefit. Clearly, more experiments will be required to understand this process fully, but certain features are already apparent.

The changes in coat colour that occurred in the first 24h after treatment, and the fact that lysyl oxidase activity of skin and caeruloplasmin oxidase activity returned to nearly normal values in samples obtained 3 days after injection, indicate that the copper was able to restore the function of at least three of the copper enzymes (tyrosinase, lysyl oxidase and caeruloplasmin) quite promptly. Presumably the copper is also effective in the cross-linking process in keratin, as evidenced by the change in the shape of the vibrissae. Further study of these processes at different times after treatment and study of other copper enzymes in treated animals will be of great interest.

Interpretation of the effects of copper therapy on growth is more difficult. Clearly the copper therapy has a beneficial effect in the long term, but the very slow growth observed in the first 16 days after treatment is a puzzle. One might attribute this to toxicity of the copper during this phase, especially since higher doses did cause frank neurological toxicity. Treated normal mice showed slower growth rates than untreated controls during the first 3 days after treatment, suggesting transient toxicity; however, growth rates of treated and untreated normal mice were identical after this time.

On examining the longer-term growth it is apparent that $Cu^+Seb(p)$ caused a significantly better outcome than any of the other forms of copper used. This finding suggests a synergistic effect between Cu^+ and sebacate. It is known that copper has to be in the Cu^+ form for incorporation into a number of copper enzymes (Österberg, 1974; Frieden & Hsieh, 1976). Copper may also penetrate membranes as Cu^+ (Österberg, 1974). Sebacic acid is known to be a

strong chelator of Cu^{2+} through its carboxy groups and is possibly a moderate complexing agent of Cu^+ through the same groups (E. G. Walliczek, unpublished work). Sebacic acid may protect Cu^+ against oxidation and may also facilitate its passage through cell membranes. It is possible that the provision of Cu^+ to intracellular processes is by-passing the fundamental defect in the mottled mutations, which might possibly involve the process which normally reduces Cu^{2+} to Cu^+ in cells, before its incorporation into proteins.

The severe toxicity of Cu–EDTA, Cu–histidine and $CuSO_4$ was surprising, since these have been the forms of copper that have generally been administered to human patients with Menkes' syndrome, and toxic effects have been described only rarely (Danks et al., 1972; Wehinger et al., 1975); however, these mice were given far more copper than any human patient has ever been given in a single day. The single subcutaneous dose was $11\,000\,\mu g$ of copper/kg body wt., whereas human dosage has generally been 200–$500\,\mu g$/kg in a single day. This subcutaneous injection causes a significant portion of the dose to build up in the skin (Table 3), thereby acting as a depot dose which probably allows a slow release of copper to tissues. Retention of ^{64}Cu at the site of injection was demonstrated in an experiment where ^{64}Cu was injected into the right thigh muscle of normal and mutant mice. After 24h, 25% of the administered dose was recovered at the injection site in the mutants compared with 15% at this site in normals. The values for left thigh muscle were 1% in mutants and 0.3% in normals. These data also provide evidence for greater retention in the mutants.

The studies of the distribution of copper in various tissues after treatment are of considerable interest, although not all the observations are immediately interpretable. They need to be considered in the light of the copper accumulation found in cells cultured from mice (Danks, 1977; Danks et al., 1978) and from humans (Goka et al., 1976; Horn, 1976; Beratis et al., 1978; Danks et al., 1978) and of kinetic studies performed with ^{64}Cu (Mann et al., 1979).

The increase in the kidney concentration of copper in mutant mice 3 days after injection is very striking. This finding is in accord with those observations in tissue culture just mentioned. Muscle and skin appear to show the same phenomenon of excessive copper content after therapy. The findings in brain, spleen and lung are less interpretable.

The findings in the liver were in striking contrast. Normal mice showed massive accumulation of copper, whereas mutants showed only a small increase from the very low concentration present in the liver before treatment. However, the fact that caeruloplasmin oxidase activity came back to normal in mutants for a period of time indicates that the copper present in the liver was available for caerulo-

plasmin synthesis. By 53 days after treatment, the liver copper concentrations of mutants and normals were similar, yet the caeruloplasmin oxidase activity of mutants was very low.

Others who have observed low liver copper concentrations in mutants after copper administration have described this as 'diminished hepatic uptake' (Hunt, 1976; Van den Hamer & Prins, 1978; Evans & Reis, 1978). However, kinetic studies with ^{64}Cu showed that the initial uptake of copper in the first 10min was identical in normal and mutant mice and that the problem really is one of diminished hepatic retention (Mann et al., 1979). From these observations it may seem that the basic defect decreases the capacity of liver cells to retain copper. This would explain the intermediate concentration of liver copper in treated heterozygote mice. However, this is the converse of the expression of the basic defect suggested above for fibroblastic cells, renal-epithelial cells and intact kidney, which is accumulation of excessive amounts of copper. This contradiction will need to be examined further, but the liver results may possibly be explained by diversion of copper to other tissues with increased ability to accumulate copper, and by excessive renal excretion. Decreased exposure of liver cells to copper in utero may have prevented development of binding sites for long-term retention.

The normal amounts of copper in mutant adult gut cells are consistent with the contention that the high concentration of copper in the lower region of the small intestine of untreated neonatal mutants is the result of pinocytosis (Camakaris et al., 1979), as this process terminates by 18 days of age (Clark, 1959).

A prolonged beneficial effect is produced by a single dose of copper in these animals despite the return of many abnormalities attributable to the failure of copper enzymes. The skin loses most of its pigmentation and the vibrissae go back to their abnormal shape. Lysyl oxidase activity is undetectable in skin at 60 days (Royce et al., 1978); however, the long life of collagen and elastin molecules may allow normality of connective tissue for many months. Apparently those components of a disease which usually cause death at 12–16 days do not recur to a serious degree. These vital effects remain to be identified. Presumably, even better results could be obtained by long-term intermittent therapy starting at 7 days or earlier.

The implications of these results for treatment of Menkes' syndrome are not yet clear. Early treatment may be crucial and may have to be given in utero to catch the human at a stage of brain development comparable with that of a 7-day-old mouse. Protection of copper in the Cu$^+$ form may be necessary.

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